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Specification and Drawing, as originally filed, with Application for Patent Serial No:
2,281,463, on August 26, 1999, by **STANLEY H. ZLOTKIN**, for "Composition
Comprising Micronutrients in Combination With Prebiotics, Probiotics, and Synbiotics".

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ABSTRACT

A composition useful for enhancing general immunity is disclosed. The composition

includes:

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- (iv) one or more micronutrients;
- (v) one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and
- (vi) a lipid-based excipient.

**COMPOSITION COMPRISING MICRONUTRIENTS IN COMBINATION WITH PREBIOTICS,
PROBIOTICS, AND SYNBIOTICS**

5 Field of the Invention

The present invention relates to supplements for enhancement of the immune system. More particularly, the present invention relates to compositions combining micronutrients, probiotics, prebiotics, and synbiotics which are especially useful for enhancement of the immune system.

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Background of the Invention

Proper nutrition is critical to the development of an effective immune system and enhancement of the natural immunosurveillance immune effector mechanism. This enhancement could be mediated either by increasing the frequency and absolute numbers of effector cells that carry out such function or by enhancement of the cellular mechanisms by which such effector cells mediate their function.

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The clinical association of particular importance is between malnutrition and an individual's ability to respond to infectious micro-organisms or their antigenic constituents. Mechanisms by which nutrition affects immunity include reduced phagocytic activity and decreased leukocyte proliferation which, respectively, result in less vigorous microbial elimination and poor clonal expansion of microbe-specific lymphocytes. In addition, cell cycle, transcription regulation, antibody production, cytokine secretion and anti-oxidant protection may also be altered. Thus, the immune problems related to nutritional deficiencies vary from increased opportunistic infections to suboptimal responses following vaccination. In such cases dietary supplementation of micronutrients is likely to enhance immune function.

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One type of malnutrition is micronutrient malnutrition, which may be defined as the insufficient dietary consumption of nutrients such as vitamin A, zinc, iron and iodine. It is a significant problem affecting more than 2 billion people worldwide, particularly women and children living in poverty.

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Iron deficiency is the most common nutritional problem in the world, affecting two thirds of children in most developing nations. Anemia resulting from iron deficiency in young children

has become very common since the level of bioavailable iron in a typical infant's diet is low while their rapid growth requires a much higher level of iron. The consequences of iron deficiency anemia (IDA) are very serious as it is associated with impaired cognitive and psychomotor development, reduced growth and decreased resistance to infection.

5 Zinc is another nutritionally essential micronutrient for humans. The zinc atom has a unique combination of properties that renders it useful in biologic systems. Zinc is an essential component of more than 200 enzymes pervading all metabolic pathways. The role of zinc in such enzymes can be structural and catalytic. Zinc is essential for cell growth and has a fundamental role in gene replication, activation, repression, transcription and translation.

10 The biologic actions of zinc have an important bearing on various components of the immune system. Zinc deficiency, both acquired and inherited, is associated with lymphoid atrophy, decreased cutaneous delayed hypersensitivity responses, lower thymic hormone activity a decreased number of antibody-forming cells and impaired T-killer-cell activity. Reduced activity of thymic hormone which is involved in the differentiation of T cells has also
15 been described in zinc deficiency.

Vitamin A is also an essential micronutrient needed in small amounts for normal functioning of the visual system, growth and development, maintenance of epithelial cell integrity, immune function, and reproduction. In the vitamin A deficient state, the human is unable to raise an adequate antibody response to bacteria and to maintain the activity and
20 number of killer cells. There is documentation, for example, that mucosal immune response to cholera toxin is impaired. Vitamin A also plays a role in the production of cell glycoprotein and in the regulation of cell division in the intestine which has a bearing on intestinal epithelial renewal during and after acute enteric infections. An association between vitamin A deficiency and increased diarrheal morbidity has been reported. Vitamin A supplementation has been shown to
25 decrease the mortality from diarrhea and measles.

Bhan et al.¹ describe the role of Zinc and Vitamin A supplementation for the prevention of diarrhea caused by malnutrition. However, this prior art reference does not disclose combining micronutrients with prebiotics and probiotics in a lipid-based excipient, in order to provide a composition which is readily administrable on addition to food.

Another factor critical to the immune system is the prevention of infection of the gastrointestinal (GI) tract. The GI tract is a dynamic and integrated ecosystem composed of an organized matrix of host cells, a fully functional immune system and numerous microbial habitats normally colonized by a diverse array of commensal bacterial species. Indigenous

5 apathogenic (non-harmful) gut bacteria occupying intestinal habitats provide the front line of mucosal defense against infection. Normal gut bacteria directly prevent intestinal colonization of pathogenic (potentially harmful) organisms by competing more successfully for essential nutrients or for epithelial attachment sites. Through the production of antimicrobial compounds, volatile fatty acids and chemically modified bile acids, indigenous gut bacteria also create a local
10 gut environment that is unfavorable for the growth of most enteric pathogens. Indeed, all animals have, and seemingly require, long-term cooperative associations with commensal bacteria in the GI tract.

During the birth process and rapidly thereafter, microbes from the mother and surrounding environment colonize the GI tract. Gut bacterial groups then undergo a
15 characteristic succession until a dense, complex, and stable microbiota has developed. Bacterial succession from that time onward, involves microbe-microbe and host-microbe interactions and is dependent on host supplied exogenous and endogenous nutrients. Thus nutritional modulation of the intestinal microbiota critically affects the susceptibility to enteric diseases and likely has long-term effects on immune competence and self-tolerance.

20 Collins et al.² discuss the role played by probiotics, prebiotics, and synbiotics in maintaining the health of the human large intestine, as well as dietary supplementation with probiotics, prebiotics, and synbiotics. However, this prior art reference does not disclose combining micronutrients with prebiotics and probiotics in a lipid-based excipient, in order to provide a composition which is readily administrable on addition to food.

25 Micronutrient malnutrition can be prevented, or at least controlled, by diet diversification, food fortification and nutrient supplementation. However, these solutions cannot readily be implemented in developing countries. For example, the ability of those in developing countries to diversify their diet is dictated not only by the availability of foods with a high nutrient content, but more importantly by the cost of such foods. Micronutrient-fortified foods are, of course, an

appropriate, effective means to prevent malnutrition; however, the cost of these foods is prohibitive to most families living in developing countries, or in developed countries, but who cannot afford these foods.

Accordingly, there is a need for a composition which combines micronutrient

- 5 supplementation with supplements for stimulation of the non-pathogenic bacterial populations in the GI tract, in order to improve general immunity.

Summary of the Invention

- 10 It is an object of the present invention to provide a composition for enhancement of general immunity in a mammal, which combines micronutrient supplements, prebiotics, probiotics, or synbiotics. The present composition advantageously provides the micronutrients in combination with one of a prebiotic, probiotic, and synbiotic in a form which is readily administrable on addition to food.

- 15 According to a first aspect of the present invention a composition useful for enhancing general immunity is provided. The composition comprises:

- (i) one or more micronutrients;
- (ii) one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and
- 20 (iii) a lipid-based excipient.

According to a second aspect of the invention, a use of the above composition is provided, wherein a therapeutically effective amount of the composition is added to food to be administered to a mammal.

- 25 Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

FIGURE 1 is a bar graph illustrating the effect of various iron-containing compositions on hemoglobin response in rats.

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Detailed Description of the Invention

The term "enhance" or "enhancing" as it is used herein refers to the development of an effective immune system through enhancement of the natural immunosurveillance immune effector mechanism. The enhancement could be mediated by either increasing the frequency and absolute number of effector cells that carry out such function or by enhancement of the cellular mechanisms by which such effector cells mediate their function. In addition, the term "enhance" or "enhancing" as it is used herein also refers to the growth of non-pathogenic bacteria in the gut in order to provide the front line of mucosal defense against infection, prevent intestinal colonization of pathogenic organisms, and create a local gut environment that is unfavorable for the growth of most enteric pathogens.

The term "micronutrient" as used herein refers to essential dietary nutrients needed by humans in small amounts. Their absence over varying periods of time will result in clinical deficiency syndromes. The preferred micronutrients for the present invention are iron, zinc, iodine, vitamin A, and vitamin C (ascorbic acid).

The term "prebiotic" as used herein refers to a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity or both of one or a limited number of bacterial species already resident in the colon³. Preferably, a prebiotic should also be:

- neither hydrolyzed by nor absorbed in the upper part of the intestinal tract;
- a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and
- able, as a consequence, to alter the colon microflora toward a more healthy composition.

Most prebiotics are directed toward the growth of lactic acid-producing organisms because of the positive effect these organisms have on the GI tract. Examples of prebiotics include but are not limited to fructooligosaccharide (FOS) (e.g. oligofructose and neosugar), inulin, GOS, lactulose, and lactitol.

5 The preferred prebiotic according to the present invention is FOS. FOS is derived from the chicory plant and is commercially available. Consumption of FOS has been shown to result in numerical predominance of bifidobacteria in feces. The following health advantages have been shown to be associated with bifidobacteria in the adult and infant human gut²:

- inhibition of pathogen growth;
- 10 • immunomodulatory activity;
- restoration of gut flora after antibiotic therapy;
- production of digestive enzymes;
- positive effects on antibiotic-associated diarrhea; and
- repression of rotaviruses.

15 The term "probiotic" as used herein refers to a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance⁴. Preferably, the present composition includes a probiotic which:

- exerts a beneficial effect on the host;
- is nonpathogenic and non toxic;
- 20 • contains a large number of viable cells;
- is capable of surviving and functioning in the gut; and
- remains viable during storage and use.

Health advantages associated with probiotic intake include^{5,8}:

- alleviation of symptoms of lactose malabsorption;
- 25 • increased natural resistance to infectious diseases of the intestinal tract;
- improved digestion; and
- stimulation of GI immunity.

Examples of probiotics include but are not limited to Lactobacilli (*L. acidophus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. reuteri*, *L. brevis*, *L. cellubiosus*, *L. curvatus*, *L. fermentum*,

L. planatarum), Gram-positive cocci (*Lactococcus lactis subsp. thermophilus*, *Enterococcus faecium*, *S. diaacetylactis*, *S. intermedius*), Bifidobacteria (*B. bifidum*, *B. adolescentis*, *B. animalis*, *B. infantis*, *B. longum*, *B. thermophilum*)

The term "synbiotic" as used herein refers to the combination use of pre- and probiotics

- 5 7. Examples of synbiotics include but are not limited to Bifidobacteria + FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

10 The term "lipid-based", as it is used herein with respect to the excipient, is meant to refer to excipients which are lipids, or which comprise a lipid component. Lipid-based excipients will combine with the micro-encapsulated iron granules of the present composition in a chemically stable manner in which no adverse interaction occurs such as undesirable aesthetic changes or undesirable changes to the taste of the product. Moreover, lipid-based excipients conveniently allow combination of the composition with foods, the means by which it is administered.

15 Preferably, one of the micronutrients included in the composition of the present invention, is iron in the form of micro-encapsulated iron granules. The micro-encapsulated iron granules of the present composition may comprise any bioavailable solid form of iron including iron salts such as ferrous sulphate, ferrous fumarate, ferrous succinate, ferrous gluconate, ferric pyrophosphate, ferric saccharate, ferric orthophosphate or any other compound capable of providing iron with an appropriate bioavailability. Bioavailability can be determined using the standard "hemoglobin-repletion" method described in detail by Fritz et al.⁸. This method
20 generally involves feeding anemic rats with a test iron compound and comparing their iron uptake with the iron uptake of anemic rats fed a reference compound determined to have a relative iron bioavailability of 100%.

25 The selected iron compound is formed into granules using techniques and machinery well-known to those of skill in the art. For use in the present composition, granules are prepared having a diameter of no more than about 850 microns. Granules of this size range can be obtained, for example, using a U.S. No.20 sieve. The granulated iron compound is provided as a fine free flowing powder.

Once formed into granules of a desired size, the iron compound is coated or encapsulated with an inert substance that will not interfere with the uptake of the iron compound

The coating functions to sustain the release of the iron, effectively masking the characteristic unpleasant taste of the iron compound, preventing discoloration of the foods to which it is added thereby providing a form of iron that can readily be added to foods. The coating also prevents the undesirable interaction between nutrients in the foods to which it is added as well as

5 additional nutrients that may be added to the composition itself. The inert coating may be selected from a number of suitable substances including, but not limited to, mono- or di-glycerides, ethyl cellulose, hydrogenated soybean oil, acacia gum and mixtures thereof.

10 The encapsulated granulated iron compound is admixed with a pharmaceutically acceptable lipid-based excipient. The term "pharmaceutically acceptable" refers to an excipient acceptable for use in the pharmaceutical and veterinary arts, which is not toxic or otherwise unacceptable. Examples of suitable lipid-based excipients include mono-, di- and tri-glycerides, especially naturally extracted unsaturated edible oils in hydrogenated form (such as vegetable oil, castor oil, cottonseed oil, corn oil, canola oil, rapeseed oil, peanut oil, sesame seed oil, coconut oil and mixtures thereof).

15 Further, the absorption of iron is known to be enhanced in the presence of reducing compounds. Examples of reducing compounds are compounds containing sulfhydryl groups such as the amino acids, lysine and histidine. The absorption of iron is also enhanced in the presence of meat. Accordingly, the present composition can advantageously be consumed with meat. Alternatively, the present composition may additionally contain desiccated meat particles
20 to provide enhanced iron absorption and to provide protein Content that would be particularly desirable for administration to populations in which protein consumption is low, such as populations in developing countries.

Preferably, the present composition is supplemented with additional micronutrients. Such additional micronutrients may function to enhance the immune system, as well as to
25 enhance the absorption of iron on administration. In a preferred embodiment of the present invention, the composition additionally comprises ascorbic acid (vitamin C), preferably in an amount ranging from about 40-50 mg per 15 mg of elemental iron. The ascorbic acid enhances the absorption of the iron into the bloodstream, providing a more effective composition. Alternatively, or additionally, the present composition may be supplemented with other

micronutrients, particularly those micronutrients which are typically absent from the diet or present in insufficient quantities. Examples of micronutrients that may be added to the composition include vitamin A, zinc and iodine, provided in appropriate bioavailable form. In this regard, vitamin A may be added to the present composition in the form of retinyl palmitate, zinc

5 may be added in the form of zinc sulfate or zinc gluconate, while iodine may be added in the form of potassium iodide. It will be appreciated that suitable amounts of additional micronutrients will vary with the micronutrient in question. For example, amounts of about 0.35-0.45 mg of retinyl palmitate per 15 mg of elemental iron, about 5-10 mg of elemental zinc per 15 mg of elemental iron and about 0.25 - 0.5 mg of iodine per 15 mg of elemental iron may
10 appropriately be added to the present composition.

A method for enhancement of general immunity in a mammal is also provided. The method involves the steps of adding a therapeutically effective amount of the present composition to a food, and then administering the food to the mammal requiring treatment. The term "therapeutically effective" as it is used with respect to the present composition refers to an
15 amount which is effective to prevent iron deficiency anemia, or at least minimize the occurrence of adverse effects related thereto, while not exceeding an amount which would be toxic or otherwise harmful. In this regard, precise dosage sizes appropriate to prevent anemia can readily be established in appropriately controlled trials. With respect to iron supplementation, it is anticipated that an effective treatment regimen will be the administration of a dosage in the
20 range of about 10 - 25 mg per day, more preferably about 10 - 17 mg per day. This dosage is applicable for administration to infants and young children, i.e. children between the ages of 2 - 5 years, as well as being appropriate for administration to older children, i.e. children above 5 years of age, and adults. Administration of larger amounts, for example, 15 -34 mg per day may be required by pregnant women.

25 It will be appreciated that there is no restriction on the foods or beverages to which the present composition can be added. Since the present composition is particularly beneficial for use in the prevention of anemia in infants and young children, the composition will typically be added to foods and beverages generally consumed by infants and young children. Examples of such foods include pureed or semi-solid foods, for example cereals, gruels, porridges, purees of

fruit, vegetables, meat or mixtures thereof, as well as milk-based products including, but not strictly limited to, milk, powdered milk, infant formula, puddings, yogurt, creamed cheese, cottage cheese, and other dairy products which form a part of the diet of infants and young children. The term milk-based products is also meant to include milk substitutes including

5 lactose-free milk and associated products, soy milk and the like.

In another aspect of the present invention, there is provided an article of manufacture including packaging material and a pharmaceutical composition contained within said packaging material which is effective to enhance general immunity. The composition comprises:

- 10 (i) one or more micronutrients;
- (ii) one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and
- (iii) a lipid-based excipient.

15 In a preferred embodiment, a single daily dosage of the composition is packaged, for example in a sachet-type package, comprising about 10-17 mg of elemental iron in the form of micro-encapsulated granules and about 400 - 450 mg of excipient. In a particularly preferred embodiment, the package will additionally include ascorbic acid in an amount of about 20-100 mg, iodine in an amount of 20-100 µg, vitamin A in an amount of 50-2500 IU, as well as prebiotics and probiotics in therapeutically effective amounts, which are known to those skilled
20 in the art.

The composition of the present invention, which combines of micronutrients with prebiotics, probiotics, and/or synbiotics in a lipid-based excipient, has an additive effect in enhancing the immune response, thereby decreasing the risk of infection.

25 The present invention is described in more detail by reference to the following specific examples which are not to be construed as limiting.

Example 1 - Preparation of an Iron-containing Composition

Encapsulated ferrous fumarate 60% (1 gram delivers 600 mg ferrous fumarate), having a particle size of no more than about 850 microns in which about 99% of the particles pass through a U.S. No.20 sieve, was obtained from Watson Foods Co., Inc. (Connecticut).

Ascorbic acid (3.5 kg; obtained from Basf) was thoroughly mixed in a large aluminum bowl with an excipient (25 kg; obtained from New Dundee Creamery, Division of Ault Foods Limited) containing corn syrup solids, hydrogenated vegetable oil and/or hydrogenated coconut oil, sodium-caseinate, potassium-phosphate di-basic, sodium phosphate di-basic, mono and diglycerides, acetylated tartaric acid esters of monoglycerides, artificial colour, and natural and artificial flavour.

In a 2-stage fill, 65 mg aliquots of encapsulated ferrous fumarate was added to foil-lined sachet packets followed by the addition of 450-500 mg of ascorbic acid/excipient mixture. The sachets were appropriately sealed along their open edge.

Optionally, 2.1 kg zinc gluconate is admixed with the ascorbic acid and excipient. This mixture is then added to ferrous fumarate-containing sachets as set out above.

Example 2 - Relative Bioavailability of Micro-encapsulated Iron

The bioavailability of iron in the composition set out in Example 1 has been determined using the hemoglobin-repletion test in rats as follows.

Male weanling Sprague-Dawley rats housed individually in stainless steel cages were fed a low iron diet and de-ionized distilled water ad lib for 24 days. The low-iron diet contained no more than about 3 mg of iron per kg of diet. Following the 24 day depletion period, approximately 200 μ l of blood was drawn from the tail vein of each rat for hemoglobin analysis. Anemic rats having hemoglobin values between 30 and 60 g/L were used in the study. The rats were housed individually in cages in a randomized block design. The rats were divided into groups, each group being fed ad libitum a test diet selected from 0, 10 or 20 mg of one of micro-encapsulated or coated ferrous fumarate (prepared as described in Example 1), micro-encapsulated or coated ferrous fumarate with zinc, uncoated ferrous fumarate particles or uncoated ferrous sulphate (a reference compound determined to have a relative bioavailability of 100) per kilogram of diet. The following chart more specifically sets out the test groups:

# of Animals	Ferrous Sulfate (Fe SO ₄ 7H ₂ O)	Coated Ferrous Fumarate	Coated Ferrous Fumarate + Zinc	Ferrous Fumarate
10	0	0	0	0
10	10 mg Fe/kg diet	0	0	0
10	20 mg Fe/kg diet	0	0	0
10	0	10 mg Fe/kg diet	0	0
10	0	20 mg Fe/kg diet	0	0
10	0	0	0 Fe; 10 mg/kg Zn	0
10	0	0	10 Fe; 10 mg/kg Zn	0
10	0	0	20 Fe; 10 mg/kg Zn	0
10	0	0	0	10 mg Fe/kg diet
10	0	0	0	20 mg Fe/kg diet
Total 100				

The results, as shown in Figure 1, indicate that hemoglobin response is dependent on the amount of iron in the rat's diet. Moreover, there was no significant difference in the hemoglobin response between rats fed similar amounts of iron as the reference compound (ferrous sulfate) versus rats fed micro-encapsulated ferrous fumarate.

Referring to Fig. 1, the control group represents rats fed a diet containing no iron, the "low iron" diet represents a diet containing 10 mg micro-encapsulated ferrous fumarate/kg of diet, the "high iron control" diet represents a diet containing 20 mg ferrous sulfate/kg of diet and the "high iron" diet represents a diet containing 20 mg micro-encapsulated ferrous fumarate/kg of diet. There was no change in the hemoglobin of the control after 14 days of feeding, while mean hemoglobin response of the low iron diet group was 18 g/L and the mean hemoglobin response of the high iron control and high iron diet groups was 31 g/L and 33 g/L, respectively.

Example 3 - Pilot Study to Determine the Efficacy of the Present Iron-containing Composition to

Prevent Anemia

Sixty infants between the ages of 6 and 12 months were recruited into the study

following parental consent, The hemoglobin of each infant was determined using a finger prick blood sample. Non-anemic infants were then randomized in a double-blind fashion to receive daily sachets containing a placebo or micro-encapsulated iron composition as prepared in Example 1.

- 5 Thirty infants will receive the placebo-sachets for 2 months, and thirty infants will receive the iron-containing sachets for 2 months. At the end of the two month period, the hemoglobin of each infant will be determined by taking a second finger prick blood sample. The difference in the number of anemic *infants* in each group will be calculated and will indicate the efficacy of the iron-containing composition.

CLAIMS:

1. A composition useful for enhancing general immunity comprising:
 - (a) ~~at least one micronutrient in a bio-available form;~~
 - 5 (b) one or more compounds selected from the group of a prebiotic, probiotic, and synbiotic, and
 - (c) a pharmaceutically acceptable lipid-based excipient.
2. The composition of claim 1, wherein the at least one micronutrient is selected from the group of iron, iodine, vitamin A, and zinc.
- 10 3. The composition of claim 2, wherein the composition comprises iron, vitamin A, and zinc.
4. The composition of claims 1-3, wherein the prebiotic is selected from at least one member of the group consisting of FOS, inulin, GOS, lactulose, and lactitol.
5. The composition of claim 4, wherein the FOS is selected from the group of
- 15 oligofructose and neosugar.
6. The composition of claims 1-3, wherein the probiotic is selected from at least one member of the group consisting of Lactobacilli, Gram-positive cocci, and Bifidobacteria.
7. The composition of claim 6, wherein the Lactobacilli is selected from at least one member of the group consisting of *L. acidophus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. reuteri*, *L. brevis*, *L. cellobiosus*, *L. curvatus*, *L. fermentum*, *L. planatarum*.
- 20 8. The composition of claim 6, wherein the Gram-positive cocci is selected from at least one member of the group consisting of *Lactococcus lactis* subsp. *thermophilus*, *Enterococcus faecium*, *S. diacetylactis*, *S. intermedius*.
9. The composition of claim 6, wherein the Bifidobacteria is selected from at least
- 25 one member of the group consisting of *B. bifidum*, *B. adolescentis*, *B. animalis*, *B. infantis*, *B. longum*, *B. thermophilum*.
10. The composition of claims 1-9, wherein the synbiotic is selected from at least one member of the group consisting of Bifidobacteria + FOS, Lactobacilli + lactitol, and Bifidobacteria + GOS.

11. The composition of claims 1-10, wherein the excipient is in an edible oil in hydrogenated form.

12. Use of the composition of claim 1-11 for enhancing the general immunity of a mammal.

5 13. Use of the composition of claim 12, wherein a therapeutically effective amount of the composition is added to food to be administered to the mammal.

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Figure 1



